CLAIMS

We claim,

1. A method for isolating a fragment of DNA containing an intermediate tandem repeat sequence using hybridization selection, comprising the steps of:

(a) providing a plurality of fragments of DNA, wherein at least one DNA fragment contains an intermediate tandem repeat sequence, a region of the DNA fragment which contains at least one repeat unit consisting of a sequence of five (5), six (6), or seven (7) bases repeated in tandem at least two (2) times;

(b) providing a stationary support having at least one oligonucleotide associated therewith, wherein the oligonucleotide includes a sequence of nucleotides which is complementary to a portion of the intermediate tandem repeat sequence; and

(c) combining the plurality of fragments of DNA with the support means under conditions wherein DNA fragments including the DNA fragment containing the intermediate tandem repeat sequence hybridize to the support means.

2. The method of claim 1, wherein the plurality of DNA fragments provided in step (a) is an enriched population of DNA fragments produced by the additional steps comprising:

fragmenting a sample of DNA, thereby producing a population of DNA fragments, wherein at least one DNA fragment contains the intermediate tandem repeat sequence;

ligating a linker containing a priming sequence to at least one end of each of DNA fragment in the population of DNA fragments; and

amplifying each linker ligated fragment using an oligonucleotide primer comprising a sequence which is complementary to the priming sequence.

3. The method of claim 2, wherein the sample of DNA is double-stranded and is fragmented with at least one restriction endonuclease.

4. The method of claim 3, wherein the sample of double-stranded DNA is fragmented with the restriction enzyme *Mbo* I, and wherein the linker is an *Mbo* I linker comprising a double-stranded DNA molecule with a single-stranded overhang sequence of nucleotides complementary to an overhang sequence at each end of the *Mbo* I digested DNA fragments.

5. The method of claim 4, wherein the linker is a double-stranded DNA molecule according to formula (I):

5'-pGCGGTACCCGGGAAGCTTGG (I)
CGCCATGGGCCCTTCGAACCCTAG-5'

wherein A, G, C, and T represent nucleotides, and wherein p indicates a phosphorylated 5' end of a DNA strand.

6. The method of claim 1, comprising the additional step of releasing the DNA fragments hybridized to the support means in step (c).

7. The method of claim 6, further comprising the steps of:

cloning each DNA fragment released from the support means into a DNA vector,

transforming host cells with the cloned vectors,

identifying a transformant containing a target cloned vector containing the intermediate tandem repeat sequence, and

releasing the target cloned vector from the transformant.

8. The method of claim 1, wherein the support means provided in step (b) comprises a material capable of directly coupling with the oligonucleotide wherein the material is selected from the group consisting of a nitrocellulose, nylon, glass, silica, and latex.

9. The method of claim 1, wherein the support means provided in step (b) comprises a material capable of indirectly coupling with the oligonucleotide, wherein the first coupling agent is bound to the oligonucleotide, and a second coupling agent

is bound to the surface of the stationary support, wherein the first coupling agent and the second agent are avidin and streptavidin, or antibody and antigen.

10. The method of claim 1, wherein the support means provided in step (b) comprises a mixture of at least two different oligonucleotides

11. The method of claim 1, wherein the intermediate tandem repeat sequence is a pentanucleotide tandem repeat sequence.

12. A method for isolating a fragment of DNA containing a pentanucleotide tandem repeat sequence using hybridization selection, comprising the steps of:

(a) providing a plurality of fragments of double-stranded DNA, wherein at least one DNA fragment contains a pentanucleotide tandem repeat sequence, a region of the DNA fragment which contains at least one repeat unit consisting of a sequence of five (5) bases repeated in tandem at least two (2) times;

(b) providing a support means having at least one oligonucleotide associated therewith, wherein the oligonucleotide includes a sequence of nucleotides which is complementary to a portion of the intermediate tandem repeat sequence; and

(c) combining the plurality of fragments of DNA with the support means under conditions wherein the DNA fragment containing the pentanucleotide tandem repeat sequence and at least one other DNA fragment hybridizes to the support means.

13. The method of claim 12, wherein the plurality of DNA fragments provided in step (a) is produced by:

fragmenting a sample of double-stranded DNA with a restriction enzyme, thereby producing a plurality of DNA fragments, wherein at least one DNA fragment contains the pentanucleotide tandem repeat sequence;

ligating a linker containing a priming sequence to at least one end of each of DNA fragment in the plurality of DNA fragments; and

amplifying each linker ligated fragment using an oligonucleotide primer comprising a sequence which is complementary to the priming sequence.

14. The method of claim 12, further comprising the steps of:

releasing the DNA fragments hybridized to the support means; and

amplifying the fragments released from the support means, using the oligonucleotide primer used to amplify each linker ligated fragment prior to hybridization to the support means.

15. The method of claim 14, further comprising the steps of:

cloning each of the amplified of DNA fragments released from the support means into a DNA vector,

transforming host cells with the cloned vectors,

identifying a transformant containing a target cloned vector containing the intermediate tandem repeat sequence, and

isolating the target cloned vector from the transformant.

16. The method of claim 12, wherein the support means provided in step (b) comprises a mixture of at least two different oligonucleotides

17. A method for detecting a target intermediate tandem repeat DNA sequence having a low incidence of stutter artifacts, comprising the steps of:

(a) providing a sample of DNA having at least one target intermediate tandem repeat sequence, wherein the target intermediate tandem repeat sequence is a region of the DNA containing at least one repeat unit consisting of a sequence of five (5), six (6), or seven (7) base pairs repeated in tandem at least two (2) times; and

(b) detecting the target intermediate tandem repeat sequence in the sample of DNA, wherein an average stutter artifact of no more than 2.4% is observed.

18. The method of claim 17, wherein the target intermediate tandem repeat sequence is a perfect intermediate tandem repeat sequence.

19. The method of claim 17, wherein the target intermediate tandem repeat sequence is an imperfect intermediate tandem repeat sequence.

20. The method of claim 17, wherein the sample of DNA provided in step (a) is human genomic DNA.

21. The method of claim 17, wherein the target intermediate tandem repeat sequence of the sample of DNA provided in step (a) is amplified prior to step (b).

22. The method of claim 21, wherein the target intermediate tandem repeat sequence is amplified using at least one oligonucleotide primer, comprising a sequence which is complementary to and flanks a region of a double-stranded DNA marker containing a template intermediate tandem repeat sequence, wherein the template intermediate tandem repeat sequence is a region of the DNA marker which contains the repeat unit sequence repeated in tandem at least two (2) times, provided that the DNA marker has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

23. The method of claim 22 wherein the oligonucleotide primer used in amplifying the target intermediate tandem repeat sequence has a fluorescent label covalently attached thereto.

SUB A2

24. The method of claim 17, wherein the stutter artifact is observed in step (b) by comparing the target intermediate tandem repeat sequence detected to fragments of known length in a DNA size marker.

25. The method of claim 24, wherein an average stutter of no more than 1.1% is observed.

SUB A3

26. A method for detecting at least one target intermediate tandem repeat sequence in a DNA sample, wherein the target intermediate tandem repeat sequence is a region of the DNA sample which contains at least one repeat unit consisting of a sequence of five (5), six (6), or seven (7) base pairs repeated in tandem at least two (2) times; the method comprising the steps of:

(a) providing at least one oligonucleotide primer comprising a nucleic acid sequence which is complementary to and flanks a region of a DNA marker containing a template intermediate tandem repeat sequence, wherein the DNA marker has a sequence selected from the group of sequences consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43;

(b) providing a DNA sample comprising the target intermediate tandem repeat sequence;

(c) using the at least one oligonucleotide primer to amplify the target intermediate repeat sequence of the DNA sample; and

(d) detecting polymorphisms in the amplified target intermediate tandem repeat sequence.

27. The method of claim 26, wherein the sample of DNA provided in step (b) is a sample of human genomic DNA.

28. The method of claim 26, wherein the target intermediate tandem repeat sequence is a perfect intermediate tandem repeat.

29. The method of claim 26, wherein the target intermediate tandem repeat sequence is an imperfect intermediate tandem repeat.

30. The method of claim 26, wherein the oligonucleotide primer provided in step (a) comprises a sequence selected from one of the groups of sequences consisting of:

SEQ ID NO:44 and SEQ ID NO:45, when the DNA marker sequence is SEQ ID NO: 1;

SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO: 58, when the DNA marker sequence is SEQ ID NO:2;

SEQ ID NO:59 and SEQ ID NO:60, when the DNA marker sequence is SEQ ID NO:3;

SEQ ID NO:61 and SEQ ID NO:62, when the DNA marker sequence is SEQ ID NO:4;

SEQ ID NO:63 and SEQ ID NO:64, when the DNA marker sequence is SEQ ID NO:5;

SEQ ID NO:65 and SEQ ID NO:66, when the DNA marker sequence is SEQ ID NO:6;

SEQ ID NO:67 and SEQ ID NO:68, when the DNA marker sequence is SEQ ID NO:7;

SEQ ID NO:69 and SEQ ID NO:70, when the DNA marker sequence is SEQ ID NO:8;

SEQ ID NO:71 and SEQ ID NO:72, when the DNA marker sequence is SEQ ID NO:9;

SEQ ID NO:73 and SEQ ID NO:74, when the DNA marker sequence is SEQ ID NO:10;

SEQ ID NO:75 and SEQ ID NO:76, when the DNA marker sequence is SEQ ID NO:11;

SEQ ID NO:77 and SEQ ID NO:78, when the DNA marker sequence is SEQ ID NO:12;

SEQ ID NO:79 and SEQ ID NO:80, when the DNA marker sequence is SEQ ID NO:13;

SEQ ID NO:81 and SEQ ID NO:82, when the DNA marker sequence is SEQ ID NO:14;

SEQ ID NO:83 and SEQ ID NO:84, when the DNA marker sequence is SEQ ID NO:15;

SEQ ID NO:85 and SEQ ID NO:86, when the DNA marker sequence is SEQ ID NO:16;

SEQ ID NO:87 and SEQ ID NO:88, when the DNA marker sequence is SEQ ID NO:17;

SEQ ID NO:89 and SEQ ID NO:90, when the DNA marker sequence is SEQ ID NO:18;

SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93 and SEQ ID NO:94, when the DNA marker sequence is SEQ ID NO:19;

SEQ ID NO:95 and SEQ ID NO:96, when the DNA marker sequence is SEQ ID NO:20;

SEQ ID NO:97 and SEQ ID NO:98, when the DNA marker sequence is SEQ ID NO:21;

SEQ ID NO:99 and SEQ ID NO:100, when the DNA marker sequence is SEQ ID NO:22;

SEQ ID NO:101 and SEQ ID NO:102, when the DNA marker sequence is SEQ ID NO:23;

SEQ ID NO:103 and SEQ ID NO:104, when the DNA marker sequence is SEQ ID NO:24;

SEQ ID NO:105 and SEQ ID NO:106, when the DNA marker sequence is SEQ ID NO:25;

SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110 and SEQ ID NO:111, when the DNA marker sequence is SEQ ID NO:26;

SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114 and SEQ ID NO:115, when the DNA marker sequence is SEQ ID NO:27;

SEQ ID NO:116 and SEQ ID NO:117, when the DNA marker sequence is SEQ ID NO:28;

SEQ ID NO:118 and SEQ ID NO:119, when the DNA marker sequence is SEQ ID NO:29;

SEQ ID NO:120 and SEQ ID NO:121, when the DNA marker sequence is SEQ ID NO:30;

SEQ ID NO:122 and SEQ ID NO:123, when the DNA marker sequence is SEQ ID NO:31;

SEQ ID NO:124 and SEQ ID NO:125, when the DNA marker sequence is SEQ ID NO:32;

SEQ ID NO:126 and SEQ ID NO:127, when the DNA marker sequence is SEQ ID NO:33;

SEQ ID NO:128 and SEQ ID NO:129, when the DNA marker sequence is SEQ ID NO:34;

SEQ ID NO:130 and SEQ ID NO:131, when the DNA marker sequence is SEQ ID NO:35;

SEQ ID NO:132 and SEQ ID NO:133, when the DNA marker sequence is SEQ ID NO:36;

SEQ ID NO:134 and SEQ ID NO:135, when the DNA marker sequence is SEQ ID NO:37;

SEQ ID NO:136 and SEQ ID NO:137, when the DNA marker sequence is SEQ ID NO:38;

SEQ ID NO:138 and SEQ ID NO:139, when the DNA marker sequence is SEQ ID NO:39;

SEQ ID NO:140 and SEQ ID NO:141, when the DNA marker sequence is SEQ ID NO:40;

SEQ ID NO:142 and SEQ ID NO:143, when the DNA marker sequence is SEQ ID NO:41;

SUB A4 CONT

SEQ ID NO:144 and SEQ ID NO:145, when the DNA marker sequence is SEQ ID NO:42; and

SEQ ID NO:146 and SEQ ID NO:147, when the DNA marker sequence is SEQ ID NO:43;

31. A kit for the detection of at least one target intermediate tandem repeat sequence in a sample of DNA, wherein the target intermediate tandem repeat sequence is a region of the sample of DNA which contains at least one repeat unit consisting of a sequence of five (5), six (6), or seven (7) base pairs repeated in tandem at least two (2) times comprising:

a container which has at least one oligonucleotide primer for amplifying the at least one target intermediate tandem repeat sequence, wherein the oligonucleotide primer comprises a sequence of nucleic acids which is complementary to and flanks a region of a double-stranded DNA marker containing a template intermediate tandem repeat sequence comprising the repeat unit repeated in tandem at least two (2) times; and wherein the DNA marker has a sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43.

32. The kit of claim 31, further comprising a DNA marker.

33. An oligonucleotide primer comprising a sequence complementary to a strand of a double-stranded DNA marker flanking a template intermediate tandem repeat sequence, wherein the template intermediate tandem repeat sequence is a region of the double-stranded DNA marker which contains at least one repeat unit consisting

of a sequence of five (5), six (6), or seven (7) base pairs repeated in tandem at least two (2) times, wherein the double-stranded DNA marker sequence is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27.

34. The oligonucleotide primer of claim 33, wherein the oligonucleotide primer comprises a sequence selected from the group consisting of:

SEQ ID NO:44 and SEQ ID NO:45, when the DNA marker sequence is SEQ ID NO: 1;

SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, and SEQ ID NO: 58, when the DNA marker sequence is SEQ ID NO:2;

SEQ ID NO:59 and SEQ ID NO:60, when the DNA marker sequence is SEQ ID NO:3;

SEQ ID NO:61 and SEQ ID NO:62, when the DNA marker sequence is SEQ ID NO:4;

SEQ ID NO:63 and SEQ ID NO:64, when the DNA marker sequence is SEQ ID NO:5;

SEQ ID NO:65 and SEQ ID NO:66, when the DNA marker sequence is SEQ ID NO:6;

SEQ ID NO:67 and SEQ ID NO:68, when the DNA marker sequence is SEQ ID NO:7;

SEQ ID NO:69 and SEQ ID NO:70, when the DNA marker sequence is SEQ ID NO:8;

SEQ ID NO:71 and SEQ ID NO:72, when the DNA marker sequence is SEQ ID NO:9;

SEQ ID NO:73 and SEQ ID NO:74, when the DNA marker sequence is SEQ ID NO:10;

SEQ ID NO:75 and SEQ ID NO:76, when the DNA marker sequence is SEQ ID NO:11;

SEQ ID NO:77 and SEQ ID NO:78, when the DNA marker sequence is SEQ ID NO:12;

SEQ ID NO:79 and SEQ ID NO:80, when the DNA marker sequence is SEQ ID NO:13;

SEQ ID NO:81 and SEQ ID NO:82, when the DNA marker sequence is SEQ ID NO:14;

SEQ ID NO:83 and SEQ ID NO:84, when the DNA marker sequence is SEQ ID NO:15;

SEQ ID NO:85 and SEQ ID NO:86, when the DNA marker sequence is SEQ ID NO:16;

SEQ ID NO:87 and SEQ ID NO:88, when the DNA marker sequence is SEQ ID NO:17;

SEQ ID NO:89 and SEQ ID NO:90, when the DNA marker sequence is SEQ ID NO:18;

SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93 and SEQ ID NO:94, when the DNA marker sequence is SEQ ID NO:19;

SEQ ID NO:95 and SEQ ID NO:96, when the DNA marker sequence is SEQ ID NO:20;

SEQ ID NO:97 and SEQ ID NO:98, when the DNA marker sequence is SEQ ID NO:21;

SEQ ID NO:99 and SEQ ID NO:100, when the DNA marker sequence is SEQ ID NO:22;

SEQ ID NO:101 and SEQ ID NO:102, when the DNA marker sequence is SEQ ID NO:23;

SEQ ID NO:103 and SEQ ID NO:104, when the DNA marker sequence is SEQ ID NO:24;

SEQ ID NO:105 and SEQ ID NO:106, when the DNA marker sequence is SEQ ID NO:25;

SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110 and SEQ ID NO:111, when the DNA marker sequence is SEQ ID NO:26; and

SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114 and SEQ ID NO:115, when the DNA marker sequence is SEQ ID NO:27.